

**HYDROPHOBIC MOIETIES IN CATIONS, ANIONS, AND ALCOHOLS PROMOTE
THE B-TO-Z TRANSITION IN POLY[d(G-C)] AND POLY[d(G-m⁵C)]**

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SUMMARY: Previous results from our laboratory showed that an increase in the nonpolar alkyl chain length in tetraalkylammonium cations is accompanied by greater efficiency in driving the B-to-Z transition. Analogous effects are observed when the hydrocarbon portion of carboxylate anions and alcohols is increased in size. The more hydrophobic species have a greater ordering effect on the aqueous solvent and promote formation of the less hydrated Z-DNA conformer.

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The lefthanded double helical conformation known as Z-DNA appears to occur in regions of alternating guanine-cytosine sequences in plasmids and chromosomes (1-3), where it is in equilibrium with the major conformation, righthanded B-DNA. The formation of Z-DNA may be involved in gene regulation (1). The *in vitro* B-to-Z transition in the synthetic DNA double helices of poly[d(G-C)] and its methylated analog poly[d(G-m⁵C)] occurs in the presence of high concentrations of 1:1 electrolytes and organic cosolvents (1,4,5). Characterization of solvent conditions that affect the B-Z equilibrium will enhance our knowledge of the relevant thermodynamic factors and contribute to a fuller understanding of the biological role of Z-DNA.

Some of the observed salt dependence of the B-to-Z transition can be explained by a greater requirement for countercations to screen DNA phosphate anions, whose mutual repulsion is stronger in the narrower Z-DNA double helix (6). However, the high salt concentrations involved suggest that changes in solvent structure also are a factor (4,7). Crystallographic data (8-11) show that Z-DNA binds less water of hydration along the sugar-phosphate backbone than does B-DNA. Thus high concentrations of salts may drive the B-to-Z transition partly by competing with DNA for water of hydration and favoring the less hydrated Z-DNA (10,12). The effect of organic cosolvents, whose addition decreases water activity, could be explained in the same fashion.

Previous work in our laboratory (12) showed a correlation among different salts between the relative solvent structure-forming tendency of the salt cation and the salt's ability to drive the B-to-Z transition. For example, increasing the alkyl chain length in symmetrical tetraalkylammonium cations

creates a larger hydrophobic surface around which water molecules cluster. It was found that halides of the larger tetraalkylammonium cations were more effective at inducing the formation of Z-DNA. In the present investigation the hypothesis linking hydrophobic hydration of organic cations to the stabilization of Z-DNA is supported by analogous trends observed with carboxylate anions and alcohols. Unlike cations, anions and alcohols cannot influence the B-Z equilibrium by neutralization of DNA phosphates. Therefore, the effect of the latter species on the B-to-Z transition provides a still clearer indicator of the importance of solute-solvent interactions that affect DNA hydration.

MATERIALS AND METHODS

1) Preparation of DNA solutions. Highly polymerized poly[d(G-C)] and poly[d(G-m⁵C)] were purchased from Pharmacia LKB Biotechnology. Salts (Gold Label or highest available purity) and alcohols (Spectrophotometric Grade) were obtained from Aldrich or Kodak. Lyophilized polymers were extensively dialyzed against 5 mM Tris·HCl, 50 mM NaCl, 2 mM Na₂EDTA, pH 7.0 to remove divalent metal contaminants and the final buffer composition was 5 mM Tris·HCl, pH 7.0 or 5 mM Tris·HCl, 50 mM NaCl, pH 7.0. Solutions for spectroscopic analysis were prepared by mixing appropriate volumes of concentrated stock solutions. Alcohol concentrations were corrected for volume changes upon mixing with the aqueous solution. Final polymer concentrations were 40-50 μM, based on a molar extinction coefficient at 260 nm of 6500 M⁻¹cm⁻¹ for the B-DNA forms (7). Mixtures were kept at room temperature for between 6 and 48 hours before spectra were recorded.

2) Spectroscopic Analysis. Circular dichroism (CD) measurements were made at ambient temperature with a JASCO J-600 spectropolarimeter interfaced to an IBM PC-XT computer. Spectra were corrected by subtraction of a solvent spectrum and subjected to a 9-point smoothing routine.

RESULTS

Table 1 summarizes our previous observations (12) on the effects of tetraalkylammonium salts on the B-to-Z transition in poly[d(G-C)] and poly[d(G-m⁵C)]. Comparison of the bromides, for example, shows that an increase in alkyl chain length is accompanied by a general decrease in transition midpoint concentration, indicating that the larger, more hydrophobic cations are more effective at stabilizing Z-DNA. The effects of tetramethyl and tetraethylammonium salts with different anions have now been compared. The results in Table 1 show a significant influence of the anion in determining a salt's ability to drive the B-to-Z transition, consistent with previous comparisons of different sodium salts (4,13) and with the study of carboxylates reported below.

To test the effect of hydrophobic moieties in organic anions on the B-to-Z transition a series of sodium salts of carboxylic acids were added to solutions of poly[d(G-C)] and poly[d(G-m⁵C)]. Sample CD spectra obtained with different concentrations of sodium propanoate are shown in Figure 1. Low salt

TABLE 1. B-to-Z transition midpoint concentrations in tetraalkylammonium salt solutions

Cation	Anion	poly[d(G-C)]	poly[d(G-m ⁵ C)]
(CH ₃) ₄ N ⁺	F ⁻	3.1	1.8
	Cl ⁻	3.4	2.0
	Br ⁻	>3.5*	2.0
	CH ₃ COO ⁻	2.7	1.8
(C ₂ H ₅) ₄ N ⁺	Br ⁻	2.9	2.1
	CH ₃ COO ⁻	2.6	1.6
(n-C ₃ H ₇) ₄ N ⁺	Br ⁻	2.4†	1.4
(n-C ₄ H ₉) ₄ N ⁺	Br ⁻	2.0	1.4

Salt concentrations are in M. Conditions are as described in Materials and Methods.

*Higher salt concentrations could not be employed because of limited solubility.

†Estimated from extrapolated curve.

spectra closely resemble those obtained at low sodium chloride concentrations and are diagnostic for B-DNA. Increasing the salt concentration produces inverted spectra similar to those observed at high sodium chloride concentrations, indicating a transition to Z-DNA (1). The cooperativity of the B-to-Z transition is demonstrated by the narrow range of concentrations used in Figure 1 and by the sigmoidal variation in ellipticity at 260 nm with salt concentration (Figure 2). The transition in poly[d(G-m⁵C)] occurred at much lower salt concentrations than in poly[d(G-C)], consistent with results using other salts (12,14,15). All of the sodium carboxylates except sodium methanoate were more effective than sodium chloride in driving the B-to-Z transition of either polymer, as evidenced by the lower transition midpoint concentra-

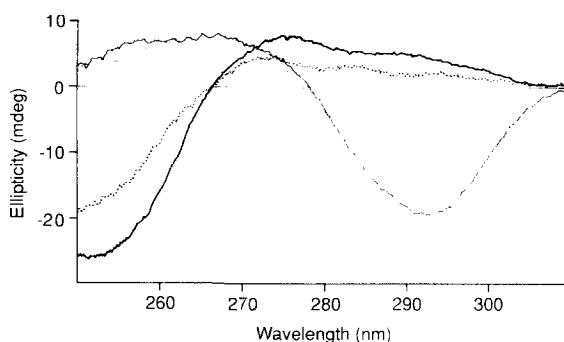


Fig. 1: CD spectra of polynucleotide duplexes in CH₃CH₂COONa solutions. Solvent also contained 5 mM Tris-HCl, pH 7.0. Spectra recorded for poly[d(G-C)] in 1.8 M (....) and 2.0 M (— · —) salt; poly[d(G-m⁵C)] in 0.6 M (—) and 0.8 M (— —) salt. All spectra are expanded on the ellipticity scale by a factor of 2.0.

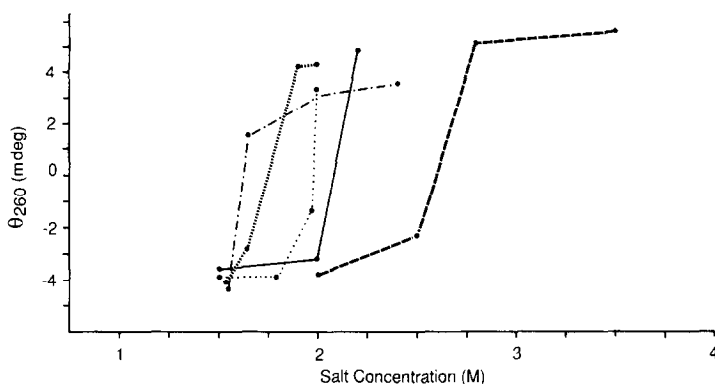


Fig. 2: Ellipticity at 260 nm of poly[d(G-C)] in sodium carboxylate solutions. Ellipticity values were obtained from CD spectra (original scale) recorded under conditions described in Fig. 1. Salt anions were HCOO^- (---), CH_3COO^- (—), $\text{CH}_3\text{CH}_2\text{COO}^-$ (.....), $\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^-$ (- · - · -), and $(\text{CH}_3)_3\text{CCOO}^-$ (- - - -).

tions for the former (Table 2). Similarly, the ethanoate salts of tetramethylammonium and tetraethylammonium were more effective than most of the respective halides (Table 1). The poly[d(G-C)] transition midpoint of 2.1 M sodium ethanoate compares closely to the value determined by Wells and coworkers (13). Although they reported highly amplified CD spectra, attributed to the formation of condensed ψ -DNA, between 2.2 and 2.7 M sodium ethanoate, our preparations yielded slightly perturbed Z-DNA-like spectra in the same concentration range (data not shown).

Figure 2 and Table 2 show that as the length of the carboxylate anion was increased from one to four carbons, the poly[d(G-C)] midpoint concentration decreased, indicating increasing effectiveness of the salt. The dimethylpropanoate anion, with five carbons in a branched chain, was slightly more effective than the four-carbon straight chain butanoate (Figure 2). With poly[d(G-m⁵C)] the larger carboxylate anions were more effective than methanoate, but no further change in midpoint concentration was observed from ethanoate to dimethylpropanoate (Table 2).

TABLE 2. B-to-Z transition midpoint concentrations in sodium carboxylate solutions

Anion	poly[d(G-C)]	poly[d(G-m ⁵ C)]
Cl^-	2.2	0.8
HCOO^-	2.6	0.8
CH_3COO^-	2.1	0.7
$\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^-$	1.8	0.7
$(\text{CH}_3)_3\text{CCOO}^-$	1.8	0.7

Sodium salt concentrations are in M. Conditions are as described in Materials and Methods and in Figure 1.

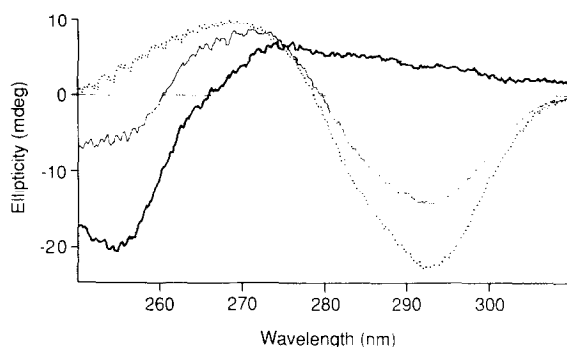


Fig. 3: CD spectra of poly[d(G-m⁵C)] in aqueous ethanol solutions. Solvent also contained 5 mM Tris·HCl, 50 mM NaCl, pH 7.0. Ethanol concentrations (M) were 2.9 (—), 3.1 (---), and 3.2 (.....). All spectra are expanded on the ellipticity scale by a factor of 3.0.

The B-to-Z transition has previously been observed in the presence of organic cosolvents such as dimethylsulfoxide, ethanol, ethylene glycol, formamide, glycerol, and trifluoroethanol (1,5,14,16,17; B. G. Rowan and R. S. Preisler, unpublished results). The effect of cosolvent hydrophobicity was tested by comparing the water-miscible alcohols. Sample CD spectra of poly[d(G-m⁵C)] in aqueous ethanol solutions (Figure 3) show spectral changes and cooperativity similar to those of the salt-induced B-to-Z transition (Figure 1). Previous reports (5,14,16) gave similar transition midpoint ethanol concentrations for both poly[d(G-C)] and poly[d(G-m⁵C)]. Effectiveness in driving the transition of poly[d(G-m⁵C)] increased with alkyl group size, from methanol (one carbon) to 2-methyl-2-propanol (four carbons—Table 3). The beginning of a similar trend was observed in poly[d(G-C)], in that ethanol was more effective than methanol (Table 3). With the longer chain alcohols lower concentrations (below 5–6M) favored the B-DNA conformer of poly[d(G-C)]. Higher concentrations produced, instead of a B-to-Z transition, anomalous CD spectra (results not shown), perhaps indicating denaturation or aggregation of the duplex.

TABLE 3. B-to-Z transition midpoint concentrations in aqueous alcohol solutions

Alcohol	poly[d(G-C)]	poly[d(G-m ⁵ C)]
CH ₃ OH	13	4.6
CH ₃ CH ₂ OH	8.2	3.1
CH ₃ CH ₂ CH ₂ OH	—	2.6
CH ₃ CHOHCH ₃	—	2.5
(CH ₃) ₃ COH	—	2.2

Alcohol concentrations are in M. Conditions are as described in Materials and Methods and in Figure 3.

DISCUSSION

In three series of polar or ionic species with hydrophobic moieties, tetraalkylammonium cations (Table 1), carboxylate anions (Figure 2 and Table 2), and alcohols (Table 3), an increase in alkyl chain length is correlated with an increased ability to drive the B-to-Z transition. The lack of any further decrease in the poly[d(G-m⁵C)] transition midpoint concentration with carboxylates larger than ethanoate (Table 2) might be because such concentrations are too low to manifest differential anion effects on the solvent. On the other hand, the inability to detect a B-to-Z transition of poly[d(G-C)] in the presence of the longer-chain alcohols (Table 3) could reflect more drastic effects on water structure, leading to duplex aggregation or denaturation.

While divalent and multivalent cations bind tightly to defined sites on the double helix and stabilize Z-DNA in millimolar or even micromolar concentrations (14,15,18-20), the high midpoint concentrations required for the species studied here suggest a less direct mode of interaction with DNA. Although these compounds are sufficiently ionic or polar to form concentrated aqueous solutions, their nonpolar moieties appear to perturb water structure in a manner analogous to the formation of ordered hydrogen-bonded networks around dissolved hydrocarbons (21). One revealing set of physical data concerns the effects of various solutes on the dielectric relaxation time of water, a measure of the reorientation rate of water molecules in response to an alternating electric field. While the relaxation time was decreased 20% in 1 M sodium chloride compared to pure water, increases of 30%, 15%, and 35% were measured in 1 M solutions of tetraethylammonium chloride, sodium propanoate, and 1-propanol, respectively (21).

As the size of the hydrophobic moiety in an organic ion or alcohol increases, it will attract a larger cluster of water molecules, thus decreasing the availability of water to hydrate DNA. This effect is expected to shift the equilibrium in favor of the more sparsely hydrated Z-DNA conformer. In addition to the dielectric relaxation data described above, a number of physical measurements demonstrate a progressive ordering or immobilizing effect on water molecules as the alkyl chain length in tetraalkylammonium cations is increased (21,22). The behavior of poly[d(G-C)] in sodium ethanoate solutions was attributed partly to the "hydrophobic tails" of ethanoate anions (13) and the hydrophobic effect seems the most likely explanation for the greater efficiency of longer carboxylate anions in driving the B-to-Z transition (Figure 2 and Table 2). The trend is even more striking when one considers that, due to decreasing acidity within this series, the ionic strength of 1 M sodium dimethylpropanoate is lower than that of 1 M sodium methanoate (all solutions were adjusted to the same pH of 7.0). However, little difference was observed between butanoate and dimethylpropanoate.

Although the latter anion has an additional carbon, the branching of its chain presents a smaller surface area to the solvent than there would be in a straight chain five-carbon carboxylate.

Others have suggested that alcohols drive the B-to-Z transition either through a hydrophobic effect on water structure (16) or through strengthened cation-phosphate binding in a medium of lower dielectric constant (1,16,23). The present results favor the former explanation. First, the dielectric constants of alcohol-water mixtures at the poly[d(G-m⁵C)] transition midpoint decrease only about 10% from methanol to 2-methyl-2-propanol (24), while effectiveness more than doubles (Table 3). Second, the only cation in these solutions was 50 mM Na⁺. Finally, the trend in Table 3 parallels the effects of increased alkyl chain length in organic cations and anions (Tables 1 and 2), lending additional support for the role of aqueous solvent perturbation in the stabilization of Z-DNA by these species.

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